

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
9 November 2000 (09.11.2000)

PCT

(10) International Publication Number  
**WO 00/66722 A1**

(51) International Patent Classification<sup>7</sup>: **C12N 15/10**,  
C12Q 1/68, C07K 14/205, C12N 15/31, 1/19, 1/21, G06F  
17/00, C12N 15/86, C07K 16/12, A61K 48/00, 39/106,  
39/40

[FR/FR]; 5, rue Mizon, F-75015 Paris (FR). SELIG, Luc  
[FR/FR]; 6, rue Mallier, F-94120 Fontenay-sous-Bois  
(FR). RAIN, Jean-Christophe [FR/FR]; 32, Jardin  
Boieldieu, F-92800 Puteaux (FR).

(21) International Application Number: PCT/IB00/00603

(74) Agents: MARTIN, Jean-Jacques et al.; Cabinet Regime-  
beau, 26, avenue Kléber, F-75116 Paris (FR).

(22) International Filing Date: 14 April 2000 (14.04.2000)

(81) Designated States (national): CA, JP, US.

(25) Filing Language: English

(84) Designated States (regional): European patent (AT, BE,  
CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC,  
NL, PT, SE).

(30) Priority Data:  
99401066.8 30 April 1999 (30.04.1999) EP

**Published:**

- With international search report.
- With amended claims.

(71) Applicant (for all designated States except US): HYBRI-  
GENICS S.A. [FR/FR]; 180, avenue Daumesnil, F-75012  
Paris (FR).

Date of publication of the amended claims: 1 February 2001

(72) Inventors; and  
(75) Inventors/Applicants (for US only): LEGRAND, Pierre

For two-letter codes and other abbreviations, refer to the "Guid-  
ance Notes on Codes and Abbreviations" appearing at the begin-  
ning of each regular issue of the PCT Gazette.



**WO 00/66722 A1**

(54) Title: COLLECTION OF PROKARYOTIC DNA FOR TWO HYBRID SYSTEMS HELICOBACTER PYLORI PROTEIN-  
PROTEIN INTERACTIONS AND APPLICATION THEREOF

(57) Abstract: The present invention concerns collections of recombinant cell clones derived from a prokaryotic genome, more particularly from *Helicobacter pylori* genome, usable for two-hybrid systems and methods to produce such collections. The invention further relates to the identification of *H. pylori* protein-protein interactions and to the application of said collections of recombinant cell clones and said identified proteins interactions to the pharmaceutical and diagnostic field.

72. Host cell according to claim 71, wherein the host cell is a prokaryotic cell.
73. Host cell according to claim 71, wherein the host cell is an eukaryotic cell.
- 5        74. Method for producing a polypeptide according to anyone of claims 45 and 61, comprising the steps of :
- a) cultivating a host cell according to anyone of claims 71 to 73 under conditions and in culture medium allowing the growth of said host cell and the expression of said polypeptide; and
- 10      b) recovering said polypeptide directly from the culture medium or from said cultivated cell obtained in step a).
75. Purified or isolated polypeptide obtained by the method according to claim 74.
76. A method for selecting an agent capable of modulating the protein-protein interaction of a set of two polypeptides according to claim 45 comprising the steps of :
- a) cultivating a recombinant cell clone containing a reporter gene expression of which is toxic for said recombinant cell clone and transformed with two plasmids wherein :
- 15      i) the first plasmid contains a nucleic construct comprising a nucleic sequence encoding a first hybrid polypeptide containing one of said two polypeptides and a DNA binding domain ;
- 20      ii) the second plasmid contains a nucleic construct comprising a nucleic sequence encoding a second hybrid polypeptide containing the second of said two polypeptides and an activating domain capable of activating said toxic reporter gene when the first and the second hybrid polypeptides are interacting ;
- 25      on a selective medium containing the agent to be tested and allowing the growth of said recombinant cell clone when the toxic reporter gene is not activated ; and
- b) selecting agent which is capable of inhibiting the growth of the recombinant cell clone cultivated in step a).
- 30      77. A method for selecting an agent capable of modulating the protein-protein interaction of a set of two polypeptides according to claim 45 comprising the steps of :

**AMENDED CLAIMS**

[received by the International Bureau on 28 November 2000 (28.11.00);  
original claim 60 amended; remaining claims unchanged (2 pages)]

- b) a polynucleotide having the sequence identified by the reference indicated in the right column "SID®" in table III ;
- c) fragment having at least 12 consecutive nucleotides of polynucleotide of a) or b), complement thereof, and RNA corresponding to said polynucleotide ; and
- 5 d) a polynucleotide having at least 80 % identity degree after alignment to a nucleic acid sequence of a polynucleotide of a) or b) ;
- with the exception of the polynucleotides encoding the polypeptide having the sequence disclosed in the EMBL Data base document Accession number 025045.

61. Purified or isolated polypeptide selected from the group consisting of :
- 10 a) a polypeptide having an amino acids sequence identified by the reference indicated in the right column "SID®" in table II, and fragment thereof having at least 5 consecutive amino acids ; and
- b) a polypeptide encoded by a polynucleotide according to claim 59 or 60.

62. Use of a polynucleotide according to claim 60 as a primer for  
15 amplification.

63. Use of a polynucleotide according to claim 60 as a specific probe for detection.

64. Cloning or expression vector containing a polynucleotide according to anyone of claims 59 and 60.

- 20 65. Vector according to claim 64, wherein the vector is the plasmid pACTIIst, pAS2ΔΔ or pP6.

66. Vector according to claim 64, wherein the vector is the plasmid selected from the group consisting of pT25, pKT25, pUT18 and pUT18C.

- 25 67. Vector according to claim 64, wherein the vector is self replicated.
68. Vector according to claim 64 or 67, wherein the vector is a viral vector.

69. Vector according to claim 68, wherein the vector is chosen between an adenovirus, AAV, a retrovirus, a proxivirus or an herpes virus.

70. Vector according to anyone of claims 64 to 69 including elements allowing expression and/or secretion of said polynucleotide in a host cell.

- 30 71. Host cell transformed with a vector according to anyone of claims 64 to 70.